

## METHODS AND COMPOSITIONS UTILIZING QUINAZOLINONES

### CROSS-REFERENCE TO RELATED APPLICATION

*This application is a continuation of application 9/699,047, filed 10/24/00, now U.S. Patent No. 6,545,004.*

- This application claims priority under 35 USC 119(e) from U.S. Provisional Application No. 60/198,253, having Jeffrey T. Finer as the inventor, filed October 27, 1999, and titled "METHODS AND COMPOSITIONS UTILIZING QUINAZOLINONES", which is incorporated by reference herein for all purposes; it also claims priority under 35 USC 119(e) from U.S. Provisional Patent Application No. 60/213,104, having Jeffrey T. Finer et al. as inventors, filed June 21, 2000, and titled "METHODS AND COMPOSITIONS UTILIZING QUINAZOLINONES", which is incorporated by reference herein for all purposes.

### FIELD OF THE INVENTION

- This invention relates to quinazolinone derivatives which are inhibitors of the mitotic kinesin KSP and are useful in the treatment of cellular proliferative diseases, for example cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders and inflammation.

### BACKGROUND OF THE INVENTION

- Interest in the medicinal chemistry of quinazoline derivatives was stimulated in the early 1950's with the elucidation of the structure of a quinazoline alkaloid, 3-[ $\beta$ -keto-gamma-(3-hydroxy-2-piperidyl)-propyl]-4-quinazolinone, from an Asian plant known for its antimalarial properties. In a quest to find additional antimalarial agents, various substituted quinazolines have been synthesized. Of particular import was the synthesis of the derivative 2-methyl-3-o-tolyl-4-(3H)-quinazolinone. This compound, known by the name methaqualone, though ineffective against protozoa, was found to be a potent hypnotic.
- Since the introduction of methaqualone and its discovery as a hypnotic, the pharmacological activity of quinazolinones and related compounds has been investigated. Quinazolinones and derivatives thereof are now known to have a wide



ATPase activity of kinesin motor domains also can be used to monitor the effects of modulating agents. In one embodiment ATPase assays of kinesin are performed in the absence of microtubules. In another embodiment, the ATPase assays are performed in the presence of microtubules. Different types of modulating agents can be detected in the above assays. In a preferred embodiment, the effect of a modulating agent is independent of the concentration of microtubules and ATP. In another embodiment, the effect of the agents on kinesin ATPase can be decreased by increasing the concentrations of ATP, microtubules or both. In yet another embodiment, the effect of the modulating agent is increased by increasing concentrations of ATP, microtubules or both.

Agents that modulate the biochemical activity of KSP in vitro may then be screened in vivo. Methods for such agents in vivo include assays of cell cycle distribution, cell viability, or the presence, morphology, activity, distribution, or amount of mitotic spindles. Methods for monitoring cell cycle distribution of a cell population, for example, by flow cytometry, are well known to those skilled in the art, as are methods for determining cell viability. See for example, U.S. Patent Application "Methods of Screening for Modulators of Cell Proliferation and Methods of Diagnosing Cell Proliferation States," filed Oct. 22, 1999, serial number 09/428,156, hereby incorporated by reference in its entirety. <sup>now US Patent No. 6,617,115,</sup>

In addition to the assays described above, microscopic methods for monitoring spindle formation and malformation are well known to those of skill in the art (see, e.g., Whitehead and Rattner (1998), J. Cell Sci. 111:2551-61; Galgio et al, (1996) J. Cell biol., 135:399-414).

The compositions of the invention inhibit the KSP kinesin. One measure of inhibition is  $IC_{50}$ , defined as the concentration of the composition at which the activity of KSP is decreased by fifty percent. Preferred compositions have  $IC_{50}$ 's of less than about 1 mM, with preferred embodiments having  $IC_{50}$ 's of less than about 100  $\mu$ M, with more preferred embodiments having  $IC_{50}$ 's of less than about 10  $\mu$ M, with particularly preferred embodiments having  $IC_{50}$ 's of less than about 1  $\mu$ M, and especially preferred embodiments having  $IC_{50}$ 's of less than about 100 nM, and with the most preferred